

PRELIMINARY CHARACTERISATION OF POLYPHENOLIC EXTRACTS FROM GRAPE SKINS

CARACTERIZAREA PRELIMINARĂ A UNOR EXTRACTE POLIFENOLICE OBȚINUTE DIN PIELIȚELE DE STRUGURI

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Abstract. In order to characterize the polyphenolic extracts obtained from grape skins, with function in the maintenance of the metabolic equilibrium and of the human organism's state of health, it was performed the HPLC analysis (high performance liquid chromatography) through which have been identified and quantified a series of phenolic acids, stilbenes ((trans-resveratrol), certain non-hydrolysable tannins (catechin and epicatechin). In addition, it was also performed the anthocyanic profile for each polyphenolic extract. There were studied the polyphenolic extracts obtained from the grape skins of seven vine varieties, of which four were autochthon (Fetească neagră, Băbească neagră, Arcaș, Negru de Drăgășani), two varieties of international brand (Cabernet Sauvignon, Merlot) and the variety with increased resistance Chambourcine. The results obtained justify the continuation of the research regarding the biologically active properties of the polyphenolic extracts that were studied.

Key words: *Vitis vinifera*, grapes, skins, polyphenols, anthocyanins.

Rezumat. În vederea caracterizării extractelor polifenolice, obținute din pielețele de struguri, cu rol în menținerea echilibrului metabolic și a stării de sănătate a organismului uman, s-a realizat analiza HPLC (cromatografie de lichide de înaltă performanță) prin care au fost identificați și cuantificați o serie de acizi fenolici, stilbeni (trans-resveratrolul) unele taninuri nehidrolizabile (catechina și epicatechina). De asemenea, s-a realizat și profilul antocianic al fiecărui extract polifenolic. Au fost studiate extractele polifenolice obținute din pielețele de strugurii a șapte soiuri de viță de vie, dintre care patru autohtone (Fetească neagră, Băbească neagră, Arcaș, Negru de Drăgășani), două din sortimentul internațional (Cabernet Sauvignon, Merlot) și soiul cu rezistență sporită Chambourcine. Rezultatele obținute justifică continuarea cercetărilor cu privire la proprietățile biologice active ale extractelor polifenolice studiate.

Cuvinte cheie: *Vitis vinifera*, struguri, pielețe, polifenoli, antociani.

INTRODUCTION

The polyphenolic compounds constitute one of the most representative classes of secondary metabolites in plants, having an essential role in the insurance of normal growth and development of the plants, some of them contributing to the pigmentation and the defence against pathogen agents and of predators (Lapornik et al., 2005; Tsao et al., 2003; Balasundram et al., 2006).

It is known that the grapes store complex mixtures of polyphenolic

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compounds, readily accessible and relatively easy to be extracted. These compounds are found especially in the seeds and skin of grapes from where they are taken over in grape juices and wines, during the maceration process (Merton S. et.al., 2003). If the grape seeds constitute the most important source of polyphenolic compounds of polymeric type (21 - 27 GAE/g fresh vegetal mass, numbering between 60 and 70% out of the total extractable polyphenolic compounds), the grape skins contain significantly less total polyphenols and polymers, but these are more variable, being included between 0,1 și 5 mg GAE/g fresh grape skins. Polymeric polyphenols are found in variable quantity of 6–43% out of the total polyphenols from the grape skins. At the same time, it must be specified that the distribution of these compounds is not equal, varying according to the genetic, climatic factors and the development of the vegetal segments, etc. (Fregoni et al., 2004).

The research from this paper attempt to realize the characterization of certain extracts obtained from the grape skins through the identification and quantification of the most important polyphenolic compounds from their composition.

MATERIAL AND METHOD

The polyphenolic extracts have been obtained from the grape skins of certain autochone vine varieties as well as of varieties of international range (Fetească neagră, Băbească neagră, Arcaș, Negru de Drăgășani, Cabernet Sauvignon, Merlot and Chambourcine). After grinding, at dimensions of 1-2 mm, the vegetal materials were degreased with ethanol. The extraction procedure was intermittent in stationary regime at a temperature of only 30 °C, the ethyl alcohol being used as solvent in a proportion of 1/10 (vegetal material (g)/solvent (mL)). For characterization, the polyphenolic extracts have been analyzed through high performance liquid chromatography (HPLC), being identified and quantified a series of phenolic acids, stilbenes (trans-resveratrol), non-hydrolysable tannins (catechin and epicatechin), certain flavones (rutin and quercitin), as well as a series of anthocyanic compounds.

RESULTS AND DISCUSSIONS

In order to evaluate the beneficial properties of the polyphenolic extracts obtained from grape skins in the maintenance of the metabolic equilibrium and of the human organism's state of health, these were submitted to a process of preliminary characterization. Among the polyphenolic compounds, phenolic acids are the most common for vegetal extracts, these being found as mixtures: hydroxybenzoic and hydroxycinnamic acids (tab. 1 and 2).

In the case of analyzed extracts, the content of hydroxybenzoic acids varied between wide limits (tab. 1). The most important hydroxybenzoic acid, gallic acid was identified in very small quantities (1,095 – 1,514 mg/L), right below the detection limit (the case of extracts obtained from the grape skins of Fetească neagră and Băbească neagră). Although in the specialty literature it is mentioned the fact that salicylic acid is formed during the process of alcoholic fermentation, it was identified in the polyphenolic extracts obtained from grape skins in

considerable amounts, in the case of the varieties Băbească neagră (37,804 mg/L) and Chambourcine (30,971 mg/L). In the analyzed polyphenolic extracts there have also been identified in small quantities other hydroxybenzoic acids, such as: p-hydroxybenzoic acid, m-hydroxybenzoic acid and vanillic and gentisic acid.

Table 1

The hydroxybenzoic acids identified in the polyphenolic extracts obtained from the grape skins of the varieties taken study

Vine varieties	p-hydroxy benzoic acid, mg/L	m-hydroxy benzoic acid, mg/L	salicylic acid, mg/L	vanillic acid, mg/L	gallic acid, mg/L	acid siringic, mg/L	gentisic acid, mg/L
Fetească neagră	0.013	0.206	1.622	1.025	-	1.072	0.959
Negru de Drăgășani	-	0.200	1.637	0.959	1.148	5.948	0.835
Arcaș	0.088	-	0.995	1.443	1.514	0.432	0.732
Merlot	0.022	0.268	2.665	1.499	1.139	2.624	1.095
Cabernet Sauvignon	0,005	0.195	2.938	0.997	1.095	1.579	1.187
Băbească neagră	0.204	0.242	37.804	1.101	-	4.154	3.219
Chambourcine	0.077	-	30.971	0.877	1.184	2.798	2.116

Through HPLC analysis of the polyphenolic acids there have been also identified a series of hydroxycinnamic acids, respectively caffeic, p-coumaric, ferulic, sinapic and chlorogenic acid (tab. 2).

Table 2

The hydroxycinnamic acids identified in the polyphenolic extracts obtained from grape skins of varieties taken study

Vine varieties	caffeic acid, mg/L	p-coumaric acid, mg/L	ferulic acid, mg/L	sinapic acid, mg/L	chlorogenic acid, mg/L
Fetească neagră	-	5.943	0.987	0.738	3.348
Negru de Drăgășani	-	7.494	0.978	1.229	4.970
Arcaș	-	7.613	0.968	1.732	3.513
Merlot	-	6.307	1.175	1.726	3.280
Cabernet Sauvignon	-	5.989	1.106	1.205	3.282
Băbească neagră	0.532	7.420	1.774	3.273	4.019
Chambourcine	0.547	6.882	1.850	2.573	3.676

Analyzing the data, one can notice the fact that no matter the variety of which extracts were obtained, the contents in hydroxycinnamic acids does not show significant variations, the values being very close one to another. Besides the phenolic acids, in the extracts obtained from grape skin of varieties taken into account for the present study there was also identified the trans-resveratrol. From the graphical representation of the obtained data (fig. 1), one can notice a low variation of the concentration of trans-resveratrol between 3,12 and 3,73 mg/L.

Furthermore, there were highlighted some non-hydrolysable (condensed) tannins, respectively catechin and epicatechin (fig. 2). As the contents of catechin are concerned, one can notice the high range of variation, from 1,95 mg/L in the case of Fetească neagră to 27,33 mg/L in the case of Negru de Drăgășani. As epicatechin is concerned, higher values were noticed at Negru de Drăgășani (20,60 mg/L) and Arcaș (20,62 mg/L), in the case of other varieties the values being close one to another (between 11,70 and 13,64 mg/L).

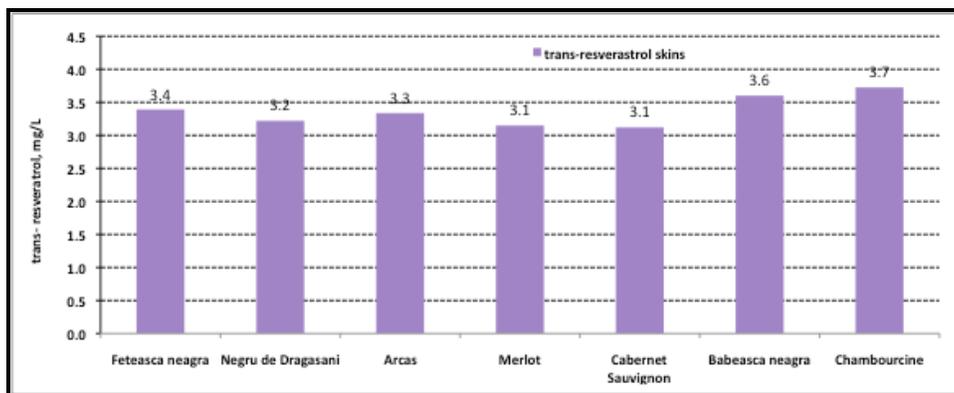


Fig. 1 - The contents of trans-resveratrol of polyphenolic extracts obtained from grape skin of varieties taken study

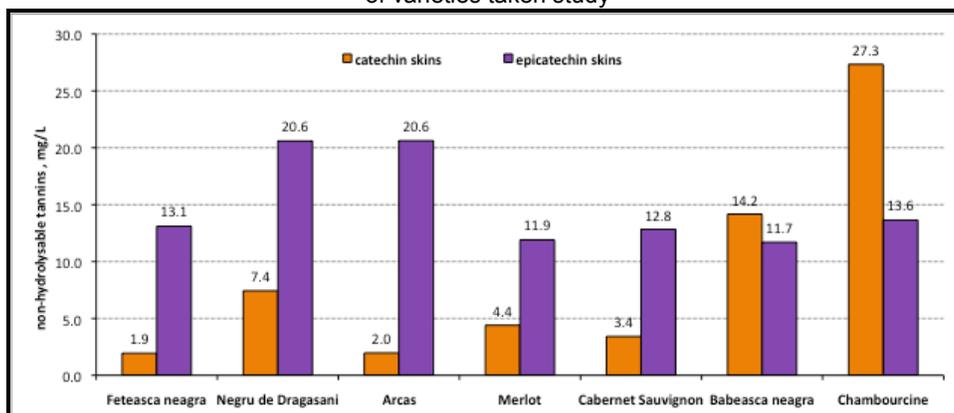


Fig. 2 – Variation of the concentration of non-hydrolysable tannins in polyphenolic extracts obtained from the grape skins of varieties study

Among flavonoids, through the HPLC analysis there were identified rutin and quercetin (fig. 3).

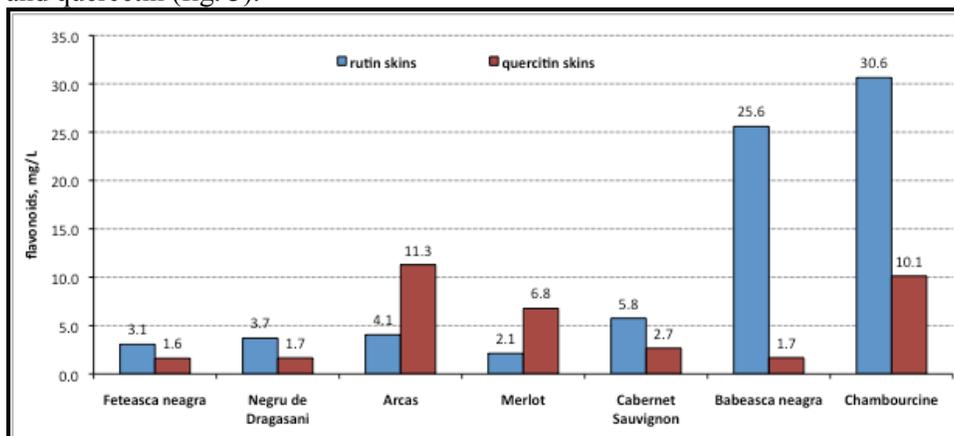


Fig. 3 – Variation of the contents of flavonoids of polyphenolic extracts obtained from grape skin of varieties taken study

Both rutin and quercetin have been identified having significant quantities. It is specially remarked the extract obtained from grape skins of the variety Chambourcine with 30,64 mg/L rutin and 10,12 mg/L quercetin.

The identification of the anthocyanic composites from polyphenolic extracts was done based on chromatograms. For each chromatogram there were identified and then calculated the relative proportions of the following anthocyanins: delphinidin-3-monoglucoside (Dp), cyanidin-3-monoglucoside (Cy), petunidin-3-monoglucoside (Pt), peonidin-3-monoglucoside (Po) malvidin-3-monoglucoside (Mv), peonidin-3-monoglucoside acetilate (Po-a), malvidin-3-monoglucoside acetilate (M-a), peonidin-3-monoglucoside coumaroylated (Po-cm), malvidin-3-monoglucoside coumaroylated (M-cm).

From the obtained chromatograms were extracted the areas of signals (in mAU*s) of anthocyanins from extracts obtained from grape skins for the seven varieties taken into account for the present study. As an evaluation of each anthocyanin from the anthocyanic extract based on areas is more difficult to achieve, the interpretation of results was done easily from the perspective of percentage proportions of areas of the main anthocyanins from extracts (tab. 3).

Table 3

Main anthocyanins (%) identified in the extracts obtained from grape skins

Main anthocyanins identified (%)	Fetească neagră	Negru de Drăgășani	Arcaș	Merlot	Cabernet Sauvignon	Băbească neagră	Chambourcine
Delphinidin	1,34	1,25	2,01	5,97	7,47	19,29	24,24
Cyanidin	1,17	1,10	1,03	3,19	3,90	14,59	17,64
Petunidin	8,41	6,67	6,48	8,09	9,41	12,56	14,42
Peonidin	21,21	13,97	11,50	19,14	13,90	9,99	7,42
Malvidină	48,87	48,25	31,99	31,24	33,64	26,62	27,27
Peonidin-acetilate	0,04	0,73	4,17	5,17	3,77	3,10	0,93
Malvidin-acetilate	2,81	5,58	21,04	16,97	19,44	9,71	4,04
Coumaroylated peonidin	3,28	3,31	4,53	3,16	1,58	0,73	0,36
Coumaroylated malvidin	12,87	19,14	17,24	7,07	6,91	3,42	3,69
ΣAnt.-acet. + Ant.-coum.	19,00	28,76	46,98	32,38	31,69	16,96	9,02
ΣAnt.-acet./ ΣAnt.-cum.	0,18	0,28	1,16	2,16	2,74	3,09	1,23

From the analysis of the results obtained it is noticed that in all extracts prevails malvidin, aspect which confirms the date in the specialty literature. A discrimination parameter in the case of black grapes can be, as in the case of wines, the sum of the esterified anthocyanins (acetylated and coumaroylated), as well as in the report between these (acetylated anthocyanins/coumaroylated anthocyanins). The sum of the acetylated and coumaroylated anthocyanins (peonidin-3-monoglucoside acetylated (Po-a), malvidin-3-monoglucoside acetylated (M-a), peonidin-3-monoglucosid coumaroylated (Po-cm), malvidin-3-monoglucoside coumaroylated (M-cm), varied between 46,98 % at the variety Arcaș and 9,02% at the variety Chambourcine. The report between acetylated anthocyanins and the coumaroylated ones presents much smaller values, which varied from 0,18% at the variety Fetească neagră to 3,09% at the variety Băbească neagră.

CONCLUSIONS

1. The identification of active principles (phenolic acids, stilbenes, tannins, flavones, etc.) of the polyphenolic extracts obtained from the grape skins contributes to the evaluation of the oenological potential of the vine varieties and, implicitly, to the evaluation of beneficial properties in the maintenance of the metabolic equilibrium and the human organism's state of health.

2. As regards the phenolic acids it was noticed that the gallic acid was identified in small quantities, between 1,095 – 1,514 mg/L, right below the detection limit in the case of extracts obtained from the grape skins of Fetească neagră and Băbească neagră. The salicylic acids presented great variation limits between 0,995 mg/L (Arcaș) and 37,804 mg/L (Băbească neagră). In reduced quantities there have also been identified other hydroxybenzoic acids, such as: *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, such as vanillic and gentisic acid. Furthermore, irrespective of the variety from which were obtained the extracts, the content of hydroxycinnamic acids (caffeic, *p*-coumaric ferulic, sinapic) did not presented significant variations, the values being very close to each other.

3. The polyphenolic extracts which were analyzed present a small variation of the *trans*-resveratrol's concentration between 3,12 and 3,73 mg/L, and a great variation range regarding the catechin from 1,95 mg/L at Fetească neagră to 27,33 mg/L at the variety Negru of Drăgășani. In addition, rutin as well as quercetin were identified in significant quantities, being remarked especially the extract obtained from the grape skins of the variety Chamburcine with 30,64 mg/L rutin and 10,12 mg/L quercetin.

4. The analysis of the anthocyanic profile highlights the fact that among all the studied extracts prevails malvidin, aspect which confirms the data from the specialty literature.

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